Evaluation of Cryopreservation Solutions based on human Platelet Lysate for Bioengineered Tissues aimed for Advanced Therapy Treatments

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Introduction and objectives

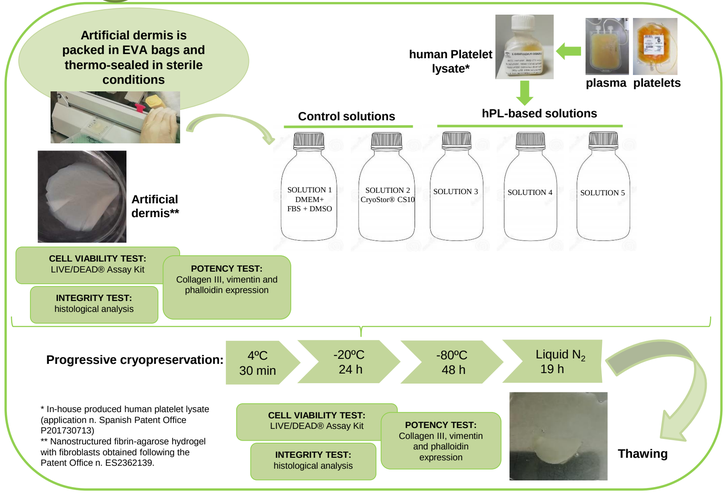
Cryopreservation is a cornerstone at the market of Advanced Therapies and Regenerative Medicine since it allows for a plausible and secure transition from manufacturing to the final treatment of patients.

To successfully cryopreserve cells, it becomes necessary to use cryoprotective agents, which preserve cellular viability in the processes of cryopreservation and during thawing. Nevertheless, tissue cryopreservation entails another burden, the preservation of the integrity of the tissue. Studies show that treatment with sugar solutions prior to cryopreservation preserve integrity. Regarding cell viability, human platelet lysate (hPL) is known as an efficient cryoprotective agent, which could substitute the presence of FBS or even reduce the concentration of DMSO in cryopreservation solutions; becoming, thus, an excellent candidate to cryopreserve cells aimed for Advanced Therapy Medicinal Products (ATMPs).

The purpose of our study is to obtain a xeno-free cryopreservation solution, based on hPL, to cryopreserve tissues for Advanced Therapies.

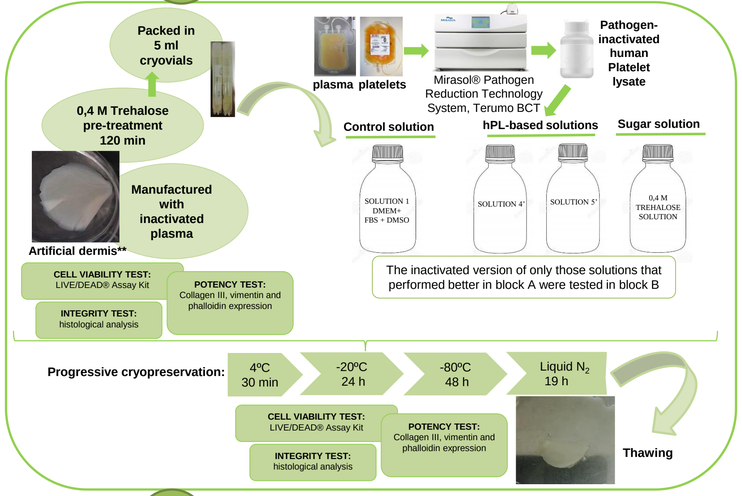
Methods

A hPL- cryopreservation solutions



* In-house produced human platelet lysate (application n. Spanish Patent Office P201730713)
** Nanostructured fibrin-agarose hydrogel with fibroblasts obtained following the Patent Office n. ES2362139.

B hPLi- cryopreservation solutions



Conclusions

hPL-based cryopreservation solutions

- Our hPL-based cryopreservation solutions maintain the viability of cells embedded in artificial dermis to a level similar to that observed in the fresh tissue.
- CryoStor® CS10 (Sol.2) and Solution 3 increment the percentage of interfibrilar spaces over the percentage observed with the pre-cryopreserved tissue.
- Our hPL-based cryopreservation solutions do not affect fibroblast potency.

hPLi-based cryopreservation solutions

- The use of inactivated plasma affects tissue integrity.
- There is a direct relation between thawing time and interfibrillar space.
- The inactivated version of our hPL-based solutions 4 and 5 (4' and 5') maintains the viability of cells embedded in the tissue with respect to pre-cryopreservation; but 0.4% trehalose solution reduced cell viability under 75%.
- Interfibrillar space measured in block B is, in general, higher than in block A.
- The solutions do not alter the functionality of the fibroblasts embedded in the artificial dermis, excepting 0.4M Trehalose.

Results

A

CELL VIAILITY TEST

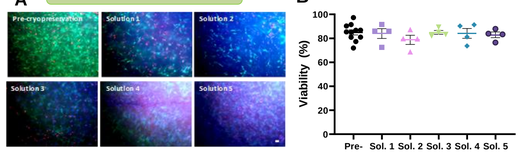


Figure 1. Cell viability test. A) LIVE/DEAD® Assay Kit staining of artificial dermis. Scale: 10 µm. B) Bar graph representation of cell viability. Statistical analysis shows there are no significant differences in cell viability between the different cryopreservation solutions after thawing compared to pre-cryopreservation. Graph shows cell viability mean and SEM.

INTEGRITY TEST

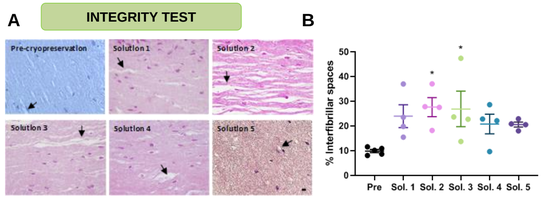


Figure 2. Histological analysis of the tissue before and after cryopreservation. A) Hematoxilin/eosin staining of tissue sections reveals interfibrillar space, arrows show pore examples. Scale: 10 µm. B) Bar graph representation of the percentage of interfibrillar spaces measured on each solution. Statistical analysis shows there is an effect of solutions in tissue integrity. Solutions 2 and 3 increment interfibrillar spaces ($p \leq 0,05$) compared to pre-cryopreservation. Graph shows measurements mean and SEM.

POTENCY TEST

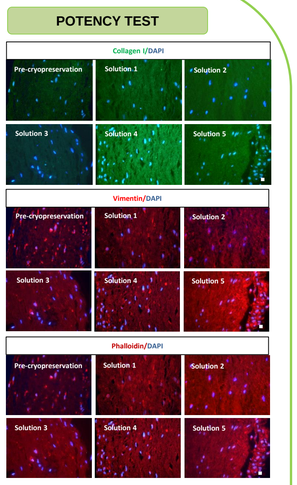


Figure 3. Fibroblast marker expression in the artificial dermis before and after cryopreservation. Cells present a similar expression of Collagen I (A), Vimentin (B) and Phalloidin (C) markers before and after cryopreservation with all solutions. The solutions do not alter cell functionality.

B

PLASMA INACTIVATION

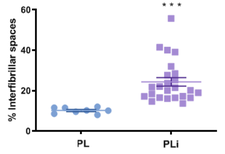


Figure 4. Comparison of interfibrillar spaces percentage prior to cryopreservation. The percentage of interfibrillar spaces in artificial dermis manufactured with inactivated plasma is significatively higher than in those manufactured with non inactivated plasma. t test: t=3,776, df=30; ***P < 0.0001

THAWING TIME

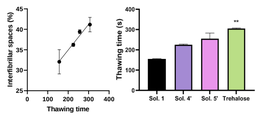


Figure 5. Thawing time influence. A) There is a direct relation between thawing time and interfibrillar spaces. B) There is a direct relation between thawing time and the cryopreservation solution employed.

CELL VIAILITY TEST

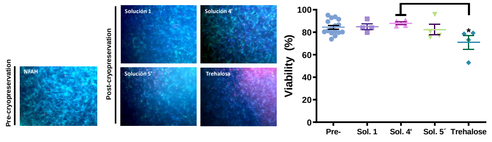


Figure 6. Cell viability test. A) LIVE/DEAD® Assay Kit staining of artificial dermis. Scale: 10 µm. B) Bar graph representation of cell viability shows hPL based solutions maintain cell viability with respect to pre-cryopreservation, but 0.4% trehalose solution significantly altered cell viability, reducing it under 75%. Graph shows cell viability mean and SEM.

INTEGRITY TEST

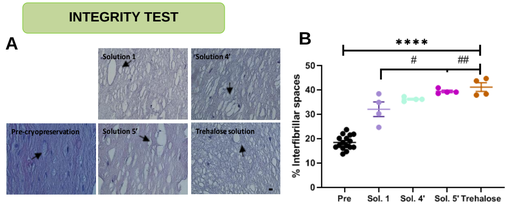


Figure 7. Histological analysis of the tissue before and after cryopreservation. A) Hematoxilin/eosin staining of tissue sections reveals interfibrillar space, arrows show some examples. Scale: 10 µm. B) Bar graph representation of the percentage of interfibrillar spaces measured on each solution. Statistical analysis shows there is an effect of solutions in the tissue integrity incrementing interfibrillar spaces comparing to pre-cryopreservation ($P < 0,0001$) there are also diferencies between solutions (#$P < 0.05$; ## $P < 0.01$). Graph shows mean and SEM.

POTENCY TEST

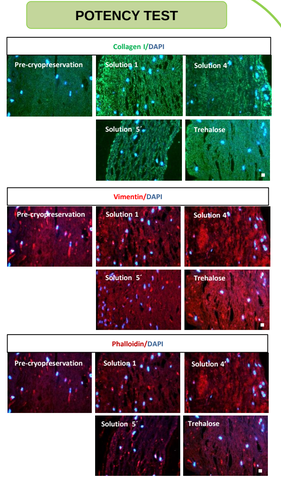


Figure 8. Fibroblast marker expression in the artificial dermis before and after cryopreservation. Cells present a similar expression of Collagen I (A), Vimentin (B) and Phalloidin (C) Markers before and after cryopreservation with all solutions. The solutions do not alter cell functionality.

References

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